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(57) Abstract

A novel relationship between pancreatico–biliary secretion and autistic syndrome is disclosed. This relationship enables a novel therapy for the treatment of the symptoms of autistic syndromes, comprising the administration of a therapeutically effective, preferably intravenous, dose of secretin to an individual with autistic syndrome. The relationship further enables a differential diagnosis for autistic syndrome, comprising an analysis of an individual's blood and/or intestinal tissue for the presence of secretin and comparison of the level of secretin to known norms.

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METHOD FOR ASSISTING IN DIFFERENTIAL DIAGNOSIS AND TREATMENT OF AUTISTIC SYNDROMES

CROSS REFERENCE TO A RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 60/047,049, filed May 19, 1997, the contents of which are incorporated herein.

FIELD OF THE INVENTION

The present invention relates in general to differential diagnosis and therapeutic treatment of autistic syndromes, and in particular to a new and useful method for diagnosing and treating autistic syndromes by measurement and administration of secretin.

BACKGROUND OF THE INVENTION

Autistic syndrome (or autism) is a pervasive developmental behavioral disorder of very early onset that is characterized by a fundamental lack of normal interest in other people. (Original description, Kanner L. Autistic disturbances of affective contact. *Nervous Child* 1943;2:217-250.) The recent diagnostic criteria (DSM IV) for autistic disorder are shown in **Table 3** below from the American Psychiatric Association[1].

Epidemiologic studies suggested a prevalence rate of autistic behavior of approximately 2 to 5 cases in 10,000, however, recent surveys including the entire spectrum of the disease indicate that rates of 15 per 10,000 are a more accurate disease prevalence[2,3]. Such figures indicate that this disorder affects four hundred thousand Americans, with significant social and public health costs.

Despite the substantial body of evidence implicating neurobiological factors in the pathogenesis, precise etiologic mechanisms of autism have yet to be identified. In the absence of a clear etiology, although both behavioral and medical interventions are available to improve learning and behavior, there is no evidence of a cure for autism, nor any efficient psychopharmacological treatments for the core symptoms.

Autism is a syndrome with multiple etiologies, as is made clear both by the evidence of neurobiological research and by the catalog of disorders that are present with autistic behaviors[4]. Based on clinical observations, there are subgroups and subtypes of subjects with significantly different patterns of strengths and deficits, different patterns of comorbidity, levels of severity, and different psychological/cognitive profiles. The response to therapeutic trials also showed a wide variety of outcomes, which may support the possibility that there are multiple etiologies for autism. Although we know that genetic, infectious, metabolic, immunologic, neurophysiological, and environmental causes may lead to similar patterns of altered development with autistic behavior, the recognition of these clear neuropathological disorders does not help us to understand the basic pathogenic mechanism of autism.

There is no clear biological marker of autism to allow early diagnosis or screening of this disease even though it is generally believed that early recognition and management is crucial in the prognosis. Under these circumstances, every clinical observation is important and may lead us to a better understanding of this disorder.

While the specific neuropathological mechanism that produces autism is unknown, it is thought to be the result of a dysfunction of particular groups of neurons in the central nervous system. The primary structures implicated in the autistic disorder are the cerebellum, cerebral cortex, and medial temporal structures. One study showed a significant loss of Purkinje cells, and to lesser extent, of granular cells in the cerebellar hemispheres of six autistic subjects[5]. Studies of two patients with autism showed that the hippocampal pyramidal neurons in the CA1 and CA4 fields displayed a decrease in dendritic branching[6]. Metabolic dysfunction of cortical areas was found through measurements by Single Photon Emission Computed Tomography (SPECT)[7]. In addition, involvement of the medial temporal lobe has been implicated by autopsy studies demonstrating increased cell density, and small cell size in the hippocampus, amygdala, enthorhinal cortex and septal nuclei[8]. An additional argument for the temporal lobe involvement is the case report describing a child with a left lateral oligodendroglioma, who fulfilled the criteria of autistic behavior[9]. This case supports the hypothesis that damage to mesial-temporal structures at

an early developmental period may lead to the autistic syndrome. Experimental evidence also supports this argument. A two-stage removal of the amygdalo-hippocampal complex in newborn monkeys resulted in behavioral changes (abnormalities of social interactions, absence of facial and body expressions and stereotypical behavior), resembling autism in children[10]. It is important to note that subgroups of autistic children displayed distinct patterns of brain activity in the frontal and temporal regions. Differences were more prominent in the left than the right hemisphere[11]. Four adult patients with autism had regionally decreased blood flow in the right lateral temporal, and in the right, left, and midfrontal lobes compared with controls[12].

The neurobiological etiology of autism is supported by the observation that epilepsy is a common concomitant of autism[13], affecting approximately one-third of adults who had childhood autism which usually had began in infancy or adolescence. In addition, different subgroups of patients have exhibited a variety of biochemical/immunological abnormalities. For example, in 20-40% of patients, whole blood serotonin levels are elevated[14], and platelet serotonin is altered. Other observations include changes in the levels of dopamine-beta-hydroxylase (DBH) in plasma[15], elevations in the levels of beta-endorphin, norepinephrine, arginine-vasopressin, and abnormally low levels of adrenocorticotropic hormone in 70% of autistic children[16], however, there are no supporting data for the autoimmune mechanism and the therapeutic trials with steroid treatment are disappointing so far.

Drug trials for autism have included tests of the effects of dopamine agonists, and antagonists to dopamine, serotonin and opiates, as well as beta blockers, ACTH analogs, and oxytocin[18]. Most of these treatments were associated with some beneficial effect in small groups of patients. The broad range of biochemical abnormalities that stimulated this wide diversity of pharmacotherapeutic trials is a clear indication that we are still far from the understanding the main pathological events in the brain resulting in autistic behavior.

Two recent hypotheses of autism are the opioid- and the immune theory. The opioid theory is based on the observation that the main features of autism are similar to features of opiate addiction. The autistic-like behavior elicited by opiate administration include: reduced socializa-

tion, affective lability, repetitive stereotyped behavior, episodes of increased motor activity, diminished crying, insensitivity to pain, and poor clinging. Motivated by this similarity, clinical trials have been conducted by using an opioid antagonist, naltrexone, in autistic patients. In an open trial, 8 to 10 children were judged to show a positive response to naltrexone[19]. However, more recent double-blinded studies found that naltrexone treatment failed to produce significant changes in social behavior[20].

Other researchers suppose that the opioids are derived from food sources. The enzymatic digest of casein and gluten contains peptides with opioid activity[21]. Fukudome and Yoshikawa isolated four opioid peptides from the digest of wheat gluten[22]. One of these peptides occurred in 15 different sites in the primary structure of glutenin, which is high molecular weight protein in wheat and considered as innocent protein in celiac disease. An additional indirect argument for the possible role of exogenous peptides was the presence of an abnormal urinary peptide pattern in patients with autism[23]. Although there is no scientific evidence that these exogenous peptides may enter the bloodstream, open clinical trials in Norway have been undertaken with the long-term elimination of gluten and casein from the diet of patients with autistic behavior and found only mild improvement[24]. As can be seen by prior research studies, while administration of opioids causes autistic behavior trials with specific and very restricted diets and opioid antagonists have not resulted in evident improvement in the behavior and health of autistic patients.

Certain immune-system abnormalities have been observed in connection with autism, such as cell-mediated immune response to human myelin basic protein[17] and changes in the percentage of different subpopulations of lymphocytes[25]. The followers of immunopathogenesis theory are trying to use large doses of steroids. The administration of steroids resulted in some improvement in the behavior of few patients. However, to maintain this improvement, continuous administration of large doses of steroid were necessary, accompanied by all the side effect of chronic steroid administration. A tapering of the therapeutic dosage of steroid resulted in an immediate relapse.

A significant portion of patients with autistic behavior also suffer from mild gastrointestinal symptoms, such as abdominal distension, constipation, or chronic loose stools. Although

these gastrointestinal problems are well known, they are not considered as important clinical features of the autistic syndromes, nor have they been treated except symptomatically. Autistic children with chronic diarrhea are not referred routinely to a pediatric gastroenterologist. In a recent study, 43% of patients had altered intestinal permeability[26], which is a strong argument for an intestinal dysfunction in a significant portion of autistic patients.

Secretin is a 27-amino acid peptide hormone produced by the S-dells of the small intestine that are spatially distributed from the upper crypt to the villus tip, being particularly numerous in the upper two-thirds of the villi[27]. The release of secretin is increased by the products of protein digestion, acid bathing, fat, sodium-oleate, bile and herbal extracts[28] (see **Figure 1A**). Secretin increases the secretion of bicarbonate in the pancreas and biliary tract, resulting in secretion of a watery, alkaline pancreatic fluid (see **Figure 1B**). The effect of secretin on the pancreas and bile duct is mediated primarily by secretin-induced elevation of cyclic AMP[29], and does not involve the inositol phosphates signal transduction pathway (see **Figure 1C**).

Secretin regulates the growth and development (enzyme composition) of the stomach, small intestine, and pancreas[30], and stimulates pancreatic fluid secretion, and bile secretion[31]. In addition, secretin has secretory, motility and circulatory effects in the gastrointestinal tract. Secretin stimulates immunoglobulin excretion through bile[32]. Secretin increases superior mesenteric blood flow, and its distribution within the mucosa and submucosa[33], as well as lymph flow[34] (see **Table 1**).

Thus far, the clinical uses of secretin are based on its secretory and vascular effects. The two most important diagnostic applications are the examination of pancreatic function, and the diagnosis of gastrinoma. There is no accepted therapeutic use. A trial to use secretin in intrahepatic cholestasis in small numbers of patients initially was encouraging[35], however, a double-blind placebo-controlled multicentric trial found no statistically significant differences in the reduction of serum bilirubin levels between secretin and placebo groups[36].

The structure of porcine secretin has been known for some time and it has been isolated from porcine intestine, and has been found to be constituted by a peptide composed of 27 amino acid residues[37]. Moreover, it has been found that bovine and porcine secretins are identical but that they are markedly different from chicken secretin[38]. Although bovine and porcine secretins behave identically with human secretin in some respects they are not structurally identical (4,806,336 Carlquist et al. 2/89). U. S. Patent No. 4,806,336 (Carlquist et al.) discloses the chemical composition of human secretin, a method for administering secretin for diagnostic use in determining pancreatic or gallbladder function, and a method for stimulating pancreatic secretion in man.

There is no published information suggesting a direct relationship between autism and secretin. However, it has been proposed that secretin and receptors of secretin are present in the brain areas that are thought to be involved in autism. Although exactly how secretin works in the brain is not yet fully understood, it seems likely that secretin regulates neurotransmitters and influences the function of a variety of cells, especially in the "hippocampal" and "amygdaloid" brain areas, where seem to be impaired in autism. (See **Table 2.**)

Our observations described in detail herein suggest that secretin is effective in the treatment of both gastrointestinal and behavioral/developmental problems in some children with autism. We observed that a group of young autistic children with chronic diarrhea, while they were undergoing tests involving an injection of secretin, had an extraordinary increase in the production of fluid from their pancreas. During the follow- up clinical visits these same children showed impressive progress in their social, behavioral and language skills, which appears so far to be permanent. We also found that the children who showed these responses to injected secretin produced only small amounts of their own secretin , and when given a dose of secretin by injection, they were able to produce an elevation in the blood level of another hormone, serotonin, which has effects on the brain.

These observations demonstrate the close relationship between secretin and serotonin in a group of autistic children. Our findings suggest that there are two subgroups of autistic patients, distinguished on the basis of gastrointestinal symptoms, their own blood secretin levels, the

increase in serotonin level after secretin injection and the quantity and quality of fluid produced for secretin stimulation. In addition, we found high prevalence of other gastrointestinal abnormalities (inflammation in the esophagus, digestive enzyme deficiencies) in children with autistic behavior which adds further support to a relationship between the presence of gastrointestinal dysfunctions and autism.

Thus, we have discovered that the gastrointestinal/brain hormone secretin has a beneficial therapeutic effect on the gastrointestinal and brain function in certain autistic children. Our findings are the first clear evidence for an association between brain and gastrointestinal dysfunctions in autistic children.

With no prior findings of secretin having the capability to influence human behavior, there has been no research into the effect of secretin on autistic disorder. This invention is based on the unique and dramatic clinical observations of the effect of secretin administered for the diagnostic evaluation of gastrointestinal function in children with autistic behavior. These observations included:

(1) significant improvement in the social communication (language) and behavioral skills; and

(2) hypersecretion of pancreato-biliary fluid in children with autism and chronic loose stools.

These observations described here open an entirely new direction in the autism research and may help to understand the pathogenesis of this disease. In addition, it may lead to a better understanding of the role of gut peptide hormones in the brain function. The existence of the gut-brain axis has been hypothesized, however, there was no clear clinical entity associated with this axis until now. This observation is the first clear evidence for an association between gastrointestinal and brain dysfunctions.

SUMMARY OF THE INVENTION

It is an object of the invention to provide a definitive method for the diagnosis of autism.

It is another object of the invention to provide an effective treatment for autism which does not require large doses of steroids or other medications.

It is a further object of the invention to provide an effective treatment for autism which does not require frequent dosage.

A particular advantage of the invention is the effectiveness of the treatment with a single dose of a non-steroidal hormone, and the continued effectiveness with follow-up doses.

These objects and others are accomplished by the stimulation of the pancreatico-biliary fluid secretion by the hormone, secretin, in a patient exhibiting autism. The release may follow the exogenous administration of an effective amount of the secretin hormone itself or, alternatively, may be subsequent to the exogenous administration of a substance that stimulates the release and/or production of secretin.

These and other objects, features, and advantages, which will be apparent from the following discussion, are achieved, in accordance with the invention, by providing a novel, therapeutically effective, preferably intravenous, dose of secretin, so as to alleviate the symptoms of autism in certain individuals suffering from autistic syndromes. Additionally, oral, intramuscular, intra-articular, intradermal, subcutaneous, inhalation, and rectal routes of administration are believed to be effective. Our observations indicate that the particular administration route is not critical to the invention.

As discussed in detail herein, the preferred means of stimulating the release of pancreatico-biliary secretion is by intravenous administration of a bolus of secretin in solution. However, alternate, less-invasive, routes of secretin application from external sources, such rectal and intradermal routes, are contemplated herein. As is known in the art, such administration would require attachment of certain biologically acceptable chemicals to assist in the mucosal or dermal absorption (know as permeation enhancers) and to protect against hydrolysis by the colonic bacterial flora or other cellular enzymes.

Alternate means of stimulating secretin release, other than exogenous administration of secretin itself, are also contemplated herein. Specifically, as previously discussed, certain agents

when delivered orally cause the body to release secretin. For example, studies have shown that a decrease in the pH of the duodenum below 4.5 results in a significant secretin release. Administration of hydrochloric acid has been shown not only to stimulate the release of secretin but also to stimulate the biosynthesis of secretin [Murthy, *Gastroenterology* 80:1237 (1981)]. Likewise, gastric acids can trigger the release of secretin. Therefore, it is clear that exogenous administration or endogenous production of acidic agents can lead to the release of secretin as well as the endogenous production of the hormone.

Other agents linked to secretin production and/or release include but are not limited to 1-phenylpentanol or 1-phenyl-1-hydroxy-N-pentane (PHP); bile salts and acids; fats and fatty acids such as sodium oleate and oleic acid; anti-ulcer compounds such as PLAUNOTOL™, tetraprenylacetone (TPN), geranyl-geranyl acetone (GGA), and (Z)-2-(4-methylpiperazin-1-yl)-1-[4-(2-phenyl-ethyl)phenyl]-eth anone oxime hydrochloride monohydrate (MCl-727); and herbal extracts such as licorice root. Thus, it is within the scope of the invention to exogenously administer a substance that can either stimulate the release of secretin or stimulate the endogenous production of the hormone.

The various features of novelty which characterize the invention are pointed out with particularity in the claims annexed to and forming a part of this disclosure. For a better under standing of the invention, its advantages and objects, reference is made to the accompanying descriptive matter in which a preferred embodiment of the invention is illustrated.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1C: Depicts the cascade of reactions related to secretin. Figure 1A depicts the intestinal phase of pancreatic secretion. Figure 1B depicts the generally accepted mechanism of bicarbonate secretion from the pancreatic duct cell. Figure 1C depicts the generally accepted mechanism of protein secretion from the pancreatic acinar cell, particularly the relationship between secretin and cAMP and phosphorylation of regulatory proteins.

Figure 2: Depicts the feed-back mechanism of secretin release.

Figures 3A-3B: Depicts the results of an Evoked Potential Response Test on Case 1 (JB) after an injection with secretin.

Figure 4: Depicts pancreatico-biliary secretion of children with autism.

Figure 5: Depicts blood secretin concentrations in autistic children.

Figure 6: Depicts changes in blood serotonin concentrations in autistic children after secretin injection.

Figure 7: Depicts secretin release after duodenal acidification in autistic children.

DETAILED DESCRIPTION OF THE INVENTION

With the exception of our data, there is not any other observation which links the intestinal peptide hormone, secretin, to the autistic syndrome. This is the first observation demonstrating a significant improvement in a neuropsychological disease after administration of a gut-peptide. Perhaps more importantly, these findings should provide the basis of new group studies that focus on the brain-gastrointestinal axis and its role in other neurological disorders. As far as the secretin is concerned, it was not considered as a clinically important neuropeptide in the brain, although several studies suggest that secretin may influence the function of the cells in the brain. Secretin receptors in the rat brain may be coupled to adenylate cyclase in a stimulatory manner[39]. Secretin injection was strikingly effective in increasing circadian rise of LH and FEB secretion in ovariectomized, estrogen-primed rats[40].

There are few studies demonstrating the presence and possible production of secretin in the brain. Secretin-like immunoreactivity (SLI) has been identified and characterized in the pituitary, hypothalamus, pineal and septum[41,42]. The relatively high concentration of secretin in the hypothalamus raises the possibility of a secretinergic pathway between the brain and the neurointermediate lobe of the pituitary[42]. The concentration in the neurointermediate lobe is about 45 fold higher than the concentration of SLI observed in the anterior lobe[42].

The existence of a possible brain-gut relation is supported by the fact that the nucleotide sequences of the coding regions of the secretin precursor RNAs (and thus the precursor proteins) produced in the small intestine are identical to those in brain and hypophysis[43]. Thus, although the role of secretin in the function of CNS has not yet been fully elucidated, it seems likely that secretin participates in neurotransmitter regulation, and influences the function of different cells (**Table 1**). Considerable data suggest that these effects are mediated via cAMP in hypothalamus and hippocampus[39].

Table 1. Physiologic Effects Of Secretin

GASTROINTESTINAL TRACT	CENTRAL NERVOUS SYSTEM
Secretory effects (stimulatory--pancreas, biliary tract)	Adenylate cyclase activation (cAMP)
Trophic effect (intestine, pancreas, stomach)	Tyrosine hydroxylase activity (increase)
Circulatory effect (selective -- intestine, pancreas)	Dopamine metabolism
Motility effect (inhibitory -- stomach, intestine)	Prolactin secretion (increase)

Regional distribution studies indicated that the specific receptor binding of secretin was greatest in the cerebellum, intermediate in the cortex, thalamus, striatum, hippocampus, and hypothalamus, and lowest in the midbrain and medulla/pons[44]. Interestingly, secretin binding was found in the regions of the brain where abnormalities were found in autism (**Table 2**).

Table 2. Localization of Secretin vs brain areas with proposed dysfunction in autism.

Brain area or cells	Autism	Secretin*				
		Binds to receptors	+SLI found	Precursor gene found	Activates adenylate cyclase	Binds to VPI receptors
Cerebellum	Yes	Yes				
Cortex	Yes	Yes				Yes
Hippocampus	Yes	Yes		Yes	Yes	Yes
Amygdaloid	Yes					
Hypothalamus		Yes	Yes		Yes	
Medulla/pons				Yes		
Hypophysis	?		Yes	Yes		
Thalamus		Yes	Yes	Yes		
Striatum		Yes				
Glioblasts					Yes	

[+SLI - Secretin-like immunoreactivity, * Not all area was examined]

Specific receptors for secretin have been characterized. Northern blot analysis of human tissue mRNA revealed that the relative intensity for expression of a 2.1-kilobase HSR transcript

was pancreas > kidney > small intestine > lung > liver, with trace levels in brain, heart, and ovary. The human secretin receptor showed a homology of 80% with the rat secretin receptor and 37% with the human type 1 vasoactive intestinal peptide receptor[45].

There are several possible explanations for the CNS effect of secretin in patients with autistic spectrum disorders:

(a) secretin receptors are present in brain areas with proposed dysfunction in autism (so far, no one has examined this possibility);

(b) secretin increases the cAMP concentration in the glioblasts[46], hypothalamus[47], paraventricular nuclei, supraoptic nucleus[48], and hippocampus (alterations in the hippocampal formation are strongly suspected in autism [49]);

(c) secretin may act by activating the VIP-1 receptors, which are predominately found in the cerebral cortex and hippocampus[50];

(d) secretin may have the same circulatory effect in the brain as in the gut, and may increase the cerebral blood flow in the area of brain containing secretin receptors such as hypothalamus, hippocampus and cortex (the comparison of pre- and post secretin SPECT studies in Case 1 showed a marked improvement in the cerebral blood flow after secretin administration, which perhaps supports such a mechanism);

(e) the hydroxylation of tyrosine to dopa is the rate-limiting reaction in catecholamine biosynthesis (it has been previously reported that secretin and other members of the secretin-glucagon family of peptides increase dopa synthesis in superior cervical ganglia in vitro[51]; it is possible that secretin influences brain catecholamine metabolism through activation of tyrosine hydroxylase); or

(f) there is a possibility of imbalance between secretin and antagonist neuropeptide hormones in the brain (for example, an imbalance between secretin and somatostatin, secretin and Peptide YY, and/or secretin and glucagon, may result in an increased or decreased sensitivity to another substance).

Secretin injected intracerebroventricularly (ICV) significantly increased defecation and decreased novel-object approaches in rats, but showed no significant effects on stereotypic behavior[52]. No autistic rat model exists in which it could be determined whether secretin relieves autistic symptoms in rats. There is no report of secretin influencing human behavior. A relationship between secretin and human behavior has never been shown or proven.

The examples contained herein are provided for illustrative purposes only, and are in no way intended to limit the scope of the present invention.

Materials and Methods

Patients

Children with autistic behavior were recruited from the Pediatric Gastroenterology and Behavioral and Developmental Pediatric Clinics at the University of Maryland. In each case, the diagnosis of autism was based on the DSM-IV criteria set forth below (**Table 3**) and was confirmed by pediatric neurologists experienced in evaluating pervasive developmental disorders.

TABLE 3. DSM-IV Criteria for Autistic Disorder

A. A total of at least six items from (1), (2), and (3), with at least two from (1), and one each from (2) and (3).

1. Qualitative impairment in social interaction, as manifested by at least **two** of the following:
   a. marked impairment in the use of multiple nonverbal behaviors, such as eye-to-eye gaze, facial expression, body postures and gestures to regulate social interaction
   b. failure to develop peer relationships appropriate to developmental level
   c. a lack of spontaneous seeking to share enjoyment, interests or achievements with other people (e.g., by lack of showing, bringing, or pointing out objects of interest)
   d. lack of social or emotional reciprocity
2. Qualitative impairments in communication, as manifested by at least **one** of the following:
   a. delay in, or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gesture or mime)
   b. in individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others
   c. stereotyped and repetitive use of language or idiosyncratic language
   d. lack of varied spontaneous make-believe play or social imitative play appropriate to developmental level.

3. Restricted, repetitive, and stereotyped patterns of behavior, interests, and activities, as manifested by at least **one** of the following:

a. Encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus

b. apparently inflexible adherence to specific, nonfunctional routines or rituals

c. stereotyped and repetitive motor mannerisms (e.g., hand or finger flapping or twisting or complex whole body movements)

d. persistent preoccupation with parts of objects

B. Delays or abnormal functioning in at least one of the following areas with onset prior to age 3 years:

1. social interaction,
2. language as used in social communication, or
3. symbolic or imaginative play

C. Not better accounted for by Rett disorder or childhood disintegrative disorder.

American Psychiatric Association, *Diagnostic and Statistical Manual of Mental Disorders, 4th ed.* 1994.

Patients were evaluated for abnormal bowel movements, evidence of reflux esophagitis, tissue activities of digestive enzymes, volume and contents of secreted pancreatico-biliary fluid after secretin stimulations, blood levels of peptide hormones and neurotransmitters and presence of intestinal bacterial or candida overgrowth. The specific procedures were as follows.

Upper gastrointestinal endoscopy and pancreatic stimulation

Chronic, non-infectious diarrhea with unclear etiology was the indication for upper gastrointestinal endoscopy. The full upper gastrointestinal work-up included biopsies for histology, measurement of the digestive enzymes of the small intestine (lactase, maltase, sucrase, glucoamylase) and the pancreas (amylase, lipase, trypsin, chymotrypsin).

After fasting from midnight, upper gastrointestinal endoscopies were carried out the next morning under general anesthesia. All gastric juice was aspirated before passing the endoscope into the duodenum. The pancreatico-biliary juice was collected after positioning the endoscope distal to the ampulla of Vater. An ERCP catheter was placed into the channel of endoscope and the fluid was collected by moving the tip into the outcoming fluid and suctioning it into a syringe. The pancreas was then stimulated with secretin 2 IU/kg BW (Ferring Laboratories, Inc, Suffern, NY, USA) given intravenously within a minute. Three additional specimens were collected after

the secretin injection within a 10-minute period. Blood samples were collected prior to and 10 minutes after secretin stimulation. In a subgroup of children, the effect of duodenal acidification was determined by measurement of blood secretin levels before and 4-5 minutes after washing the second part of the duodenum with 0.05M hydrochloric acid for 5 minutes. At the end of the procedure, biopsies were obtained of the small intestine, esophagus and stomach.

Analyses

The volume of secreted fluid was calculated as ml/min and the aspirated juice analyzed for pH, protein (mg/ml; Bio-Rad protein assay), and for enzymes (amylase, trypsin, lipase, chymotrypsin, and carboxypeptidase A and B). These enzyme assays were modified by us and run regularly in our certified Clinical Laboratory. An aliquot of collected fluid was sent for bacterial and fungal culture. Intestinal biopsy specimens were homogenized in ice-cold distilled water and the activities of lactase, maltase, sucrase, palatinase and glucoamylase were measured using the Dahlquist intestinal disaccharidases assay [Dahlquist, *Anal. Biochem,* 22:99-107 (1968); Azad M, *Pediatr Res*, 1990;28:166- 170 (1990)]. The normal values were established based on measurements of histological normal intestinal biopsy tissues (n=104) at the University of Maryland. In our practice, digestive enzyme activities below the established 3d percentile values are considered abnormal.

Examination of the biopsies of esophagus for reflux esophagitis used the following histological criteria: eosinophilic or lymphocytic infiltrate in the squamous epithelium, basal layer thickening and papillary hypertrophy. The gastric biopsies were stained with Giemsa to identify Helicobacter pylori infection.

Blood samples were analyzed for the levels of gastrointestinal peptide hormones (Secretin, CCK, VIP, Peptide Y) and neurotransmitters (Serotonin, Substance P). All the assays were performed in the Interscience Institute (Inglewood, CA) specialized in gastrointestinal hormone measurements.

Behavioral evaluation

Prior to the secretin administration, each child underwent developmental/psychological evaluation. The post-secretin evaluation was based upon the notes of therapists and teachers who did not know about the treatment, and parent interviews and videotape recordings of child behaviors. In addition, most underwent a structured evaluation including assessment of intelligence, language ability, adaptive function [Vineland Adaptive Behavior Scales][53] and behavioral rating scales [Child Behavior Checklist][54]. A more specific measure directly related to autism, the Childhood Autism Rating Scale [CARS][55], was also used.

The most recent 8 cases had developmental/psychological evaluations at the University of Maryland or at the Kennedy Krieger Institute prior to the secretin administration.

Specific Case Studies

Case 1. J.B., a 3 and 3/12 year old boy, was the product of a full-term uncomplicated pregnancy. Development of language and social behavior proceeded normally until about 15 months of age. At that time he lost his expressive vocabulary and his social behavior deteriorated. He was clinically diagnosed with autism by a pediatric neurologist at 1-1/2 years of age. At 2-1/2 years of age, a multidisciplinary evaluation changed the diagnosis to PDD not otherwise specified. Brainstem evoked potential studies revealed abnormal responses to frequency modulations in sound, which suggested the involvement of the temporal lobes and the thalamocortical afferents. Single photon emission computed tomography (SPECT) scan of the brain revealed decreased perfusion in the right hemisphere, with the most severe decrease in the right parietal-temporal region. Because of his chronic diarrhea and elevated antigliadin IgG antibody titer, he underwent an upper gastrointestinal endoscopy. After the administration of 2 IU/kg BW of secretin, the patient had an extraordinary pancreatic secretory response (10 ml/min). Three weeks after the procedure his mother reported significant changes in his gastrointestinal symptoms and behavior. His chronic diarrhea resolved and he became potty-trained. More dramatic changes occurred in his autistic behavior (**Table 4**). Among these were improvements in eye contact, alertness, expressive language, and fine motor skills. Eight months after the procedure he has still retained

his cognitive gains. At that time, he received a second single secretin infusion which resulted in a further improvement in his language and cognitive functions. The latest behavioral evaluation indicates that he has changed from a low functioning autistic child to a social, non-autistic, speech-delayed child. Repeat evoked potential studies indicated only a minimal delay in responses eight months after secretin administration (see **Figures 3A and 3B**). In addition, there was a less marked decrease in perfusion of right posterior parietal and right temporal lobes upon follow-up SPECT imaging when compared to the previous study.

Table 4. Changes after Secretin Stimulation in Case #1 (age 3.5 years)

Before Secretin	**Progress after Secretin administration (within two months)**
Two words	100's of words - will repeat some approximation of any word requested
No sentences	Short sentences, such as "I love you", "I want juice", "Good night mammy"
No flash cards	40-50 flash cards
No focus on requested tasks	Will sit and watch carefully. Will perform most tasks after watching once or twice. Will sort by color or category. Will construct more complicated puzzles. Will respond appropriately to "what's this?"
Diapers only	Completely potty trained
Watch videos	Now gets "involved" interactively with his videos. He will imitate the hand motions, sing the songs or dance to the music.
Consistent sleeping problems	Has slept through almost every night entirely
Infrequent (1-2 times/week) "spinning" episodes	No spinning episodes
Abnormal bowel movements	Normal bowel movements
Excessive water consumption	Excessive water consumption - no change
Limited diet preferences	No change
No apparent connections made between language and objects	Many connections made between new language learned and objects. Recites names he has learned on flash cards when he sees the same on computer game or video.
No response to request for gestures	Responds to all kinds of requests and spontaneously says these things himself.
No interest in drawing	Wants to draw constantly. Will draw complete face and name the parts as he draws.
Did not imitate commands	Will imitate almost any multi-step command
Minimal eye contact	Eye contact 75% of the time

Case 2. A.S., a 5 year old boy with autism, was referred with a two year history of diarrhea and food intolerance. His prenatal and postnatal history were unremarkable. His autism was diagnosed by a pediatric neurologist at two years and 9 months of age. According to his parents, he appeared normal and responsive until about 2 years of age, when he completely lost his speech and no longer responded to his name. He was placed on a high dose steroid therapy at age 4 with mild improvement. However, the beneficial effect diminished with a relapse during the gradual steroid discontinuation. Intravenous immunoglobulin was initiated later, but did not result in sustained benefit. During the endoscopy he received 2 IU/kg body weight of secretin. There was also a significantly increased secretory response after secretin administration (7.5 ml/min). The cognitive skills of this boy improved over a five weeks period following a single dosage of secretin, and he continues to progress (**Table 5**). He received a second dose of secretin 3 months later, which caused a further improvement in his social behavior and language.

Table 5. Changes after Secretin stimulation in case #2 (age 6 yrs).

Category	Before Secretin	Changes after Secretin	Time
Alertness, concentration	Staring into space; self-stimulation; does not pay attention	Extremely alert; looking therapist directly in the eye; responds immediately to commands	2 days
Correct responses to drills	Often guessing; not paying attention; 50% correct responses	Responds more quickly; pays better attention; 75-80% correct responses	1 week
Receptive language	Variable; understands 20-30% of commands; mostly one-step commands	Retrieves objects; follows 2-3 step commands; understands where he is going; understands 75% of commands	3 weeks
Expressive language	Rarely repeats words	Repeats 20-30% of what he hears; starting to verbalize on his own; "give me"; "come on" pointing to letters on ABC's and saying them "V", "W", "X", "Y", "Z".	4-6 weeks
Sleep	Sleeps 7-8 hours; often wakes up at night	10-12 hours per night uninterrupted every night	1 day
Fine motor	Very poor fine motor coordination	Putting together Legos; turns key in door; turns small knobs	4 weeks
Gross motor	Will climb steps; rides bike with assistance	Starts to hang on monkey bars; goes down fire pole with help; riding his bike up hill and into new areas	4 weeks
Activity level	Hyperactive even during drills; self simulations during drills and school	Very energetic but has very good focus during drills	1 week
Appetite	Variable; eats one meal per day; eats only 1-2 foods	Eating more foods; 2-3 large meals per day; increased variety of foods; wanted to eat dairy	1 week
Social interaction	Avoid interactions, except with parents	Hugging therapists; better eye contact, improved mood	1 week
Bowel movements	Sometimes loose, pale stools	At first constipated for 2-3 days, then normal dark brown stools	2-3 days
Movements	On toes, abnormal hand and arm positions; flexion of left arm; motor tics	A decrease in most abnormal movements but still has hyperactivity, especially in the hands	1 week

Case 3. D.T., a 4 year old boy, was referred for chronic diarrhea with foul smelling stools which were positive for blood. Autistic Disorder was diagnosed at age 2-1/2. He had significant delays in speech and cognitive development with limited social skills. Because of his chronic diarrhea with foul smelling stools, he was referred to an allerologist, and multiple food allergies were diagnosed. He was placed on a significantly restricted diet, without any

improvement in the consistency of his stools. He was also not potty-trained. He underwent an upper gastrointestinal endoscopy, and his pancreato-biliary response to secretin was excessive with an output of 8 ml/minute. Histological study revealed mild reflux esophagitis. The culture of his duodenal fluid for candida and bacteria was negative. His chronic diarrhea resolved. Most of the "claimed" antigenic foods were reintroduced into his diet without difficulty. The post-secretin behavioral evaluations were performed at three weeks and two months after the procedure. His language, social, and communication skills improved significantly (**Table 6**). A second infusion of secretion 3 months later accelerated his improvement in his language and communication skills.

Table 6. Changes after Secretin stimulation in case #3 (age 4 yrs)

Category	Before	Changes
Alertness, concentration	Staring into space; self-stimulation (chewed hands, clothing, objects); does not pay attention	Chewing clothing, hands, and licking have stopped. Overall more alert. he is able to stay on tasks until completion.
Correct responses to drills/commands	Often guessing; not paying attention; 50% correct responses	Rarely guessing. Very tuned in. Perhaps 80% correct responses
Cognitive	Engaged in random, aimless activities (dumping toys, clicking all over the computer screen, turning it on and off)	Actions are more purposeful. Playing sequencing, matching, and memory games on the computer. Listening to and observing stories attentively.
Receptive language	Variable; understood 50% of commands; mostly one-step commands	Understands almost everything. Understands two-step commands. Understands and is able to respond to the question: How many?
Expressive language	Rarely repeats words; one word only, two words, no sentences	Repeating almost everything. Still no sentences, but has definite interest in finding out the names of things! He tries repeatedly to make his message clear. Recognizes and reads a number of letters of the alphabet. Knows and can say numbers up to 10.
Social interaction	Shy. Slow to warm up to people. At school engaged in a lot of parallel play	Now has a friend at school. He participates actively in group activities and plays more with his brother and sister. He helps with simple household chores when asked. He shares food with sister.
Eye contact	Limited eye contact with parents, siblings	More eye contact. Seeks out attention and once engaged in activity, shows good eye contact.

Sleep	Difficulty settling down at times. Would not allow bedtime storied to be read to completion.	Now goes to bed willing and happily. Takes great interest in his bedtime stories, but settles down right away when told to do so.
Fine motor skills	Poor. Unable to use knife, scissors, crayon, mouse. Seldom using spoon or fork. He used a spoon for eating porridge, but resisted using it for other foods.	Now uses fork and spoon much more readily and accurately. Uses mouse very precisely. Starting to hold crayon better and use scissors better.
Activity level.	Hyperactive even during drills; self simulations during drills and school. When watching videos, for example, he would jump up and down and shriek with excitement.	Less hyperactive. More settled. More attentive and compliant to tasks. Will bounce in place on trampoline when needs to self regulate. When he watches videos, he imitates, and anticipates all actions, gestures and some words, clearly following and enjoying the story line.
Appetite	Was on highly restrictive diet for over a year. He ate well, but was frustrated by diet. Seemed to require enormous quantities of food to satisfy his appetite.	Normal diet seems to satisfy him more. No longer needs excessive quantities and actually leaves snacks, treats in favor of activities. He eats like a normal four year old.
Bowel movements	Loose stools or diarrhea	Normal bowel movement since week three
Movements	Jumping in response to videos, music	Now he sits down and enjoys listening to music, even hums along.

Typical pancreatic juice output in children without behavioral abnormalities is approximately 1-2 ml/minute between the ages of 1 and 6, and the adult response varies between 1.6 to 5.9 ml/minute[56,57]. The average response rate in each of the three cases reported above was 10, 7.5 and 8 ml/minute, respectively. Additionally, in each case, administration of secretin produced relief from autistic symptoms, and the second injection resulted in further improvement in their behavior and language functions.

Group Results

The number of children on whom we have collected data and/or completed analysis from specific tests varies and the N for each group is given in the graphs, tables or texts. For certain comparisons, the children were divided into two groups based on the presence or absence of chronic diarrhea.

Improved stool consistency

We evaluated seven children reported to have chronic loose stools. Interestingly, all of them had an improvement in the consistency of stools after the endoscopy and secretin injection. In five children this improvement was permanent; however, two had a relapse in their diarrhea later.

Prevalence of reflux esophagitis

We asked the parents specific questions regarding unexplained irritability or sudden aggressive behavior in their children. Six out of 15 children had these symptoms. Histological examination of 11 esophageal biopsy specimens provided confirming evidence of reflux esophagitis in 5 (45.5%). In addition, one child whose parents did not report this problem also had inflammation in the esophagus. This 45.5% incidence of esophagitis may suggest a common upper gastrointestinal motility problem in autistic children. The prevalence of reflux esophagitis in Western countries is estimated to be only 2%[58]. Children with esophagitis received at least two months treatment with prokinetic (Cisapride) and H2 blocker (Zantac) medications.

Digestive enzyme and pancreatico-biliary fluid analyses

Rate of pancreatico-biliary secretion:

The time required to collect basal duodenal fluid samples (1-2 ml) prior to secretin administration was 2-5 minutes, which was similar to that of non-autistic controls. The average stimulatory response for secretin in children, 3-7 years of age, is 1-2 ml/min based on our data by using the same endoscopic collection technique. Published adult responses using duodenal intubation and extended periods of specimen collection vary between 1.5 and 4.9 ml/min[56,57]. Six out of 7 autistic patients who had chronic loose stools had pancreatico-biliary secretion rates above 5 ml/min (**Figure 4**). It was observed that all children, after secretin injection, had an extensive pancreato-biliary secretory response when compared to non-autistic patients. The highest rate of secretion was 10ml/min in one child.

pH, protein and enzymes:

The pH of the collected fluid varied between pH 6 and 8.8. The protein content of basal samples was between 0.27 mg/ml and 2.46 mg/l and for samples collected immediately after secretin injection varied between 0.58 and 2.9 mg/ml. The protein content of final samples (due to the dilution effect) was between 0.62 and 1.79 mg/ml.

All of the duodenal fluid enzyme activities were within the normal range based on assays performed in our Laboratory on specimens from 215 children without pancreatic disease, i.e., cystic fibrosis.

Disaccharidases and glucoamylase

The intestinal brush border membrane enzymes -disaccharidases and glucoamylase- were measured in 12 children. Four children each had one abnormal enzyme activity and in 1 boy the activities of two enzymes were low (**Table 7**). In summary, abnormal levels in at least one of the carbohydrate digestive enzymes was found in 5/12 children (41.6%). The most frequent abnormality was hypolactasia (abnormally low lactase level).

Table 7. Abnormal disaccharidase and glucoamylase enzyme activities in autistic children

	Lactase	Maltase	Sucrase	Palatinase	Glucopamylase
Below 3rd percentile	3	1	0	1	1

Culture of the duodenal fluid.

Four out of the 15 children who underwent endoscopic procedure had urine tests for organic acid and the results suggested that they have intestinal yeast overgrowth. Duodenal fluid specimens from 11 children were tested for both bacteria and fungi (Candida) and the tests for all the patients were normal, including those with positive urine organic acid tests.

Gastrointestinal hormone and serotonin blood levels

Blood concentrations prior to and after secretin injection

The average basal secretin level in blood was significantly lower in children with chronic diarrhea ($p<0.024$) (**Figure 5**). Interestingly, the basal serotonin level after 9-12 hours of fasting was normal in all patients (N=16), however, ten minutes after secretin injection, children with chronic diarrhea showed a significant elevation in their blood serotonin level (**Figure 6**). We repeated this test in two children with the highest elevations and they showed the same degree of response in their blood serotonin level after secretin administration. Elevated serotonin levels have been reported in approximately 30% of patients with autism[59-65]. However, it is not clear from these papers whether the serotonin was measured after fasting or following meals. While there is no change in the blood serotonin levels in healthy adult volunteers after meals[66], our data showing that a certain fasting autistic children have increased serotonin levels after secretin injection may indicate that the serotonin levels should be measured in the fasting state in all autistic patients. It is possible that the reported increased blood serotonin levels were the consequence of a secretin release after meals. Our finding suggest that there may be two subgroups of patients with autistic behavior based on the serotonin response to secretin injection and possibly to meals as well. The relevance of this finding to the etiology of autism and to the familiar occurrence of hyperserotonemia in family members needs to be addressed in the future. There was no change at all and no subgroup differences in the blood levels of VIP, substance P or CCK after secretin injection. Peptide Y (measured in four children) showed no abnormality in blood levels either before or after the secretin administration.

Blood secretin concentrations after duodenal acidification

The normal physiologic process of secretin release from the intestinal S-(secretin) cells has been outlined earlier (Figures 1-2). We examined this response in 5 children during endoscopy by measuring secretin release following the acid washing of the duodenum. This acidification, which decreases pH to below 4.5, should release secretin into the blood.

Figure 7 shows the secretin levels in blood before and after 4-5 minutes of acidification. Although the sample number did not allow a clear conclusion it appears that children with low base secretin level less likely release secretin than children whose base level was in the normal range.

Based on our findings, we propose there are two subgroups of autistic patients , distinguished on the basis of gastrointestinal symptoms, fasting blood secretin levels, and secretory responses to either duodenal acidification or secretin injection (**Table 8**).

Table 8. Subgroups of patients based on the secretin and serotonin measurements

	Group I	Group II
Main gastrointestinal symptoms	Chronic loose stools/diarrhea	No diarrhea
Basal secretin level	Low (<12pg/ml)	Normal (>12 pg/ml)
Pancreatic secretion	Increased (>5 ml/min)	Normal (<4 ml/min)
Response to duodenal acidification	,2 pg/ml elevation of secretin	>2 pg/ml elevation of secretin
Serotonin level after secretin injection	>50% elevation	<50% elevation

Behavior Evaluations

The number and type of evaluation techniques that were employed increased across subjects as did the degree of sophistication of the professional staff involved in the design and conduct of the evaluation approaches. Specifically, data on the first seven subjects consisted of brief clinical observations, parental report, analysis of videotapes, and reports of evaluations by professionals who had conducted routine assessments not specifically dictated by our research protocol. Whereas, data on the second eight subjects studied consisted of the same methods described above, as well as with the addition of direct observation of behavior employing a standard functional analysis of structured videotape samples (analyzed in random order by blind raters); standard assessments including Communication and Symbolic Behavior Scales (CSBS), Pre-School Language Scales - 3 (PSLS), Bayley Scales of Infant Development: Second Edition, Vineland Adaptive Behavior Scales, Childhood Autism Rating Scale (CARS), and Behavior Observation System (BOS). Further, the evaluation team for the last eight subjects involved professionals from the areas of neuropsychology, behavioral psychology and

developmental pediatrics from both the University of Maryland School of Medicine and the Kennedy Krieger Institute/Johns Hopkins University School of Medicine.

The findings on all fifteen subjects are truly exciting. For example, in terms of parental report and non-protocol dictated evaluations, dramatic changes in cognitive, social, language, and age appropriate skills were reported for four of the first seven subjects studied. For one child (JB) - a 3-year, 3-month-old boy presenting with chronic diarrhea and autistic symptoms, including no eye contact and no social interaction - significant changes occurred in both his gastrointestinal symptoms and behavior within three weeks after the procedure. Specifically, his chronic diarrhea resolved and he was able to be potty trained. Dramatic changes occurred in his autistic behavior in terms of a dramatic improvement in eye contact, alertness, expressive language and fine motor skills. Follow-up eight months after the procedure indicated that he had retained these improvements, especially with regard to cognitive skills. Most impressively, the latest formal evaluation indicates that his diagnosis has changed from a low functioning autistic child to a social, non-autistic, speech delayed child. A second case (AS) - an older boy (5-years, 9-months of age) with chronic diarrhea and autistic symptomatology first noticed at two years of age - demonstrated progressive improvement in cognitive skills over the five-week period following the administration of secretin. Similarly, the third case (DT) - a 4-year, 3-month-old boy with chronic diarrhea, evidence of blood in the stool, reports of allergies to seventeen food substances, autistic symptoms including severe aggression, no social interaction, and highly distractable attention - showed post-secretin changes including significant improvements in language, social, and communication skills. The last of the four cases (DP) - a 3-year, 10-month-old boy with chronic diarrhea, no speech, no eye contact, and a significant sleep disorder - improved after the secretin injection in that he was able to be potty trained within three weeks and showed significant improvement in language skills in general and expressive language gains in particular.

In terms of the last eight subjects studied, in addition to parental reports comparable to the first four successes, some of the standardized test scores for this last group of subjects indicated quite impressive gains. One child (WB) - a 3 year, 1 month-old boy with autistic symptomatology but without gastrointestinal symptoms, showed a 37% increase within four

months on the CSBS scale as well as an increase in language skills equivalent to five months to one year in development as measured by the PSLS and lastly, an 11 point increase on the Bayley Scale of Development. The second child (SG) - 3 years, 10 month-old boy, who presented with chronic diarrhea, but in terms of autistic symptomatology was one of the highest functioning children studied, showed evidence of impressive gains, including a 25% increase over a period in the CSBS, as well as six-to-nine month increases in language skills, and an increase in all five areas tested on the Vineland Scale of Adaptive Behaviors. A third subject (JP) - 3 year, 9 month-old boy with chronic diarrhea presented with a variety of autistic symptomatology, including poor eye contact, no social interactions, self-stimulatory behavior, and delayed echolalia; but after the administration of secretin his markedly high activity level was decreased by 50% as measured by actometers and he showed an increase on the BOS of 22%. The last case described here showed both initial, small improvements and later quite dramatic changes post secretin administration; specifically, this case (BA) - 3 years, 8 month-old boy with chronic loose stools presented with autistic symptoms of solitary play, echolalia, and poor social interaction; whereas immediately after secretin administration, a 22% increase in the scores on the Vineland Scale of Adaptive Behavior was noted as well as a delayed reaction reported by the parents some one-to-two months later that included improved eye contact, increased focus on tasks, as well as improvements in language.

In summary, more than half of the children who received secretin were found to have reductions in autistic symptoms and positive changes in cognitive, language, and social skills that were considerably greater than that which would be expected from developmental maturation. This novel relationship between pancreato-biliary secretion and autistic disorder enables a novel therapy for the treatment of the symptoms of autistic syndromes, comprising the administration of a therapeutically effective, preferably intravenous, dose of secretin to an individual suffering from autistic spectrum disorders. The relationship further enables a differential diagnosis for autistic syndrome, comprising an analysis of an individual's pancreatic response, or blood/intestinal biopsy specimen, for the presence of secretin and comparison of the levels to known norms.

We hereby incorporate by reference our article published in January 1998 in the *Journal of the Association for Academic Minority Physicians* entitled "Improved Social and Language Skills in Patients with Autistic Spectrum Disorders Following Secretin Administration." In addition, all references cited herein are incorporated by reference in their entirety.

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While a specific embodiment of the invention has been shown and described in detail to illustrate the application of the principles of the invention, it will be understood that the invention may be embodied otherwise without departing from such principles, and that various

modifications, alternate constructions, and equivalents will occur to those skilled in the art given the benefit of this disclosure. Various changes and modifications can be made therein without departing from the spirit and scope of the invention. Thus, the invention is not limited to the specific embodiment described herein, but is defined by the appended claims.

WHAT IS CLAIMED:

1. A method for aiding in the diagnosis of autism comprising the steps of drawing a blood sample from a patient suspected of suffering from autism, analyzing the level of secretin, and correlating said level with norms.

2. A method for alleviating the symptoms of autism in an individual exhibiting symptoms of autism, comprising the step of stimulating the secretion of pancreatico-biliary fluid.

3. A method as in Claim 2 wherein said step of stimulating the secretion of said pancreatico-biliary fluid stimulates the endogenous release of serotonin in said individual.

4. A method as in Claim 2 wherein said step of stimulating the secretion of said pancreatico-biliary fluid comprises the step of administering an effective amount of secretin to said individual.

5. A method as in Claim 4 wherein the amount of secretin administered is between about 2 IU of secretin per kg of said individual's body weight.

6. A method as in Claim 4 wherein the method of administration is selected from the group consisting of intravenous, oral, intramuscular, intra-articular, intradermal, subcutaneous, inhalation, and rectal.

7. A method as in Claim 4 wherein said secretin is chosen from the group consisting of synthetic or recombinant human secretin, porcine secretin and bovine secretin.

8. A method as in Claim 2 wherein said step of stimulating pancreatico-biliary fluid secretion comprises the step of releasing endogenous secretin in said individual.

9. A method as in Claim 8 wherein said step of releasing endogenous secretin comprises the step of administering an effective amount of acid to the duodenum.

10. A method of Claim 8 wherein said step of releasing endogenous secretin comprises the step of administering an effective amount a secretin release stimulating substance, said substance selected from the group consisting of bile salt; bile acids; 1-phenyl-1-hydroxy-N-pentane (PHP); fats; fatty acids; anti-ulcer pharmaceuticals and herbal extracts.

11. A method of Claim 10 wherein said substance is sodium oleate.

12. A method of Claim 10 wherein said substance is an oleic acid.

13. A method of Claim 10 wherein said substance is an anti-ulcer pharmaceutical.

14. A method of Claim 13 wherein said anti-ulcer pharmaceutical is selected from the group consisting of PLAUNUTOL™, tetraprenylacetone (TPN), geranyl-geranyl acetone (GGA), and (Z)-2-(4-methylpiperazin-1-yl)-1-[4-(2-phenyl-ethyl)phenyl]-eth anone oxime hydrochloride monohydrate (MCl-727).

15. A method of claim 10 wherein said substance is an herbal extract.

16. A method of claim 15 wherein said herbal extract is licorice root.

17. A method of Claim 10 wherein said substance is a bile salt or bile acid.

18. A method of Claim 10 wherein said substance is 1-phenylpentanol or 1-phenyl-1-hydroxy-N-pentane (PHP) or a derivative thereof.

19. A method of Claim 4 wherein said secretin is co-administered with a biologically acceptable permeation enhancing agent.

20. A method of Claim 4 wherein said secretin is co-administered with a biologically acceptable agent capable of preventing hydrolysis by colonic bacterial flora or cellular enzymes.

FIGURE 1A:

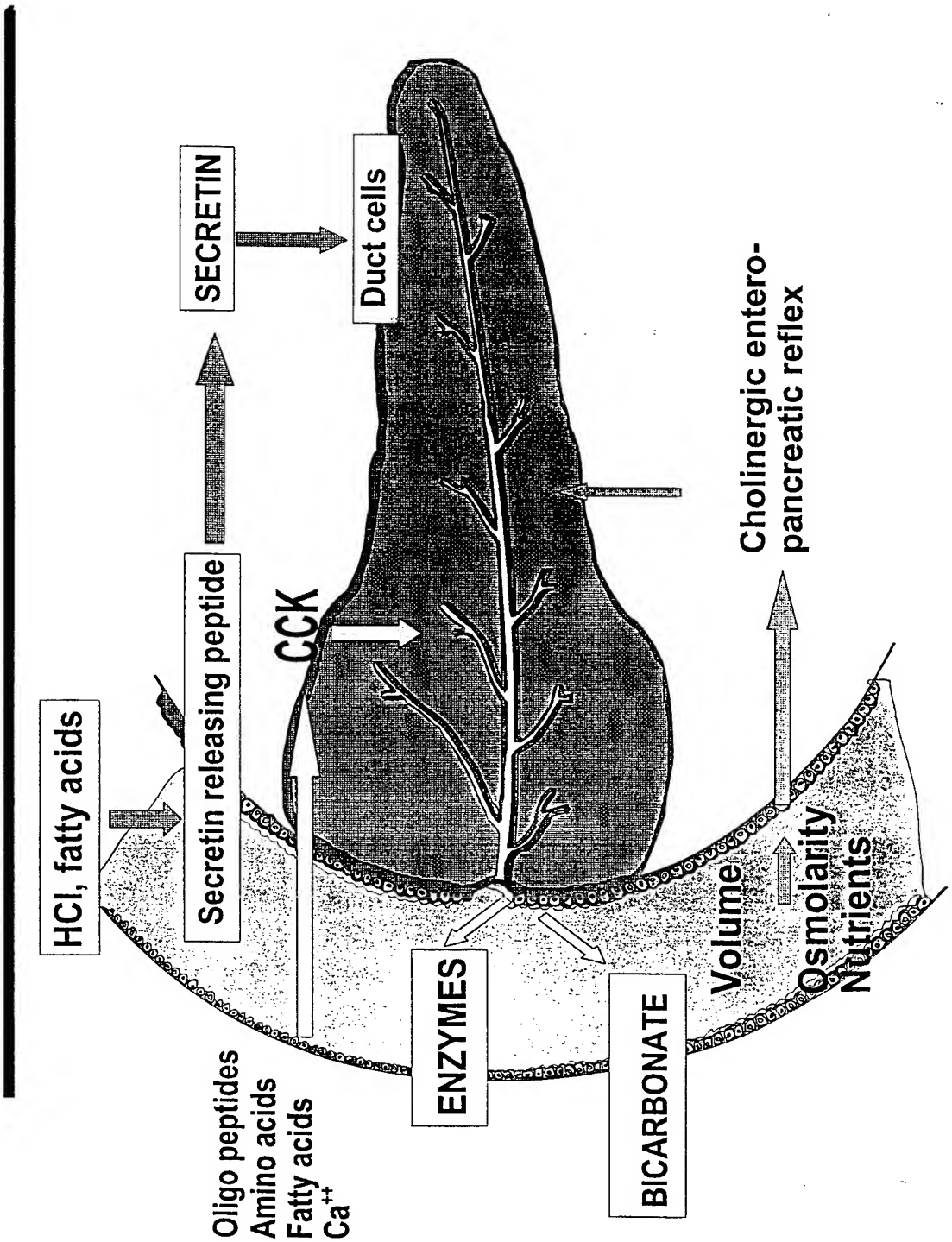

INTESTINAL PHASE OF PANCREATIC SECRETION
HCl, fatty acids
Secretin releasing peptide
SECRETIN
Duct cells
CCK
Cholinergic entero-pancreatic reflex
Oligo peptides
Amino acids
Fatty acids
Ca++
ENZYMES
BICARBONATE
Volume
Osmolarity
Nutrients

FIGURE 1B:

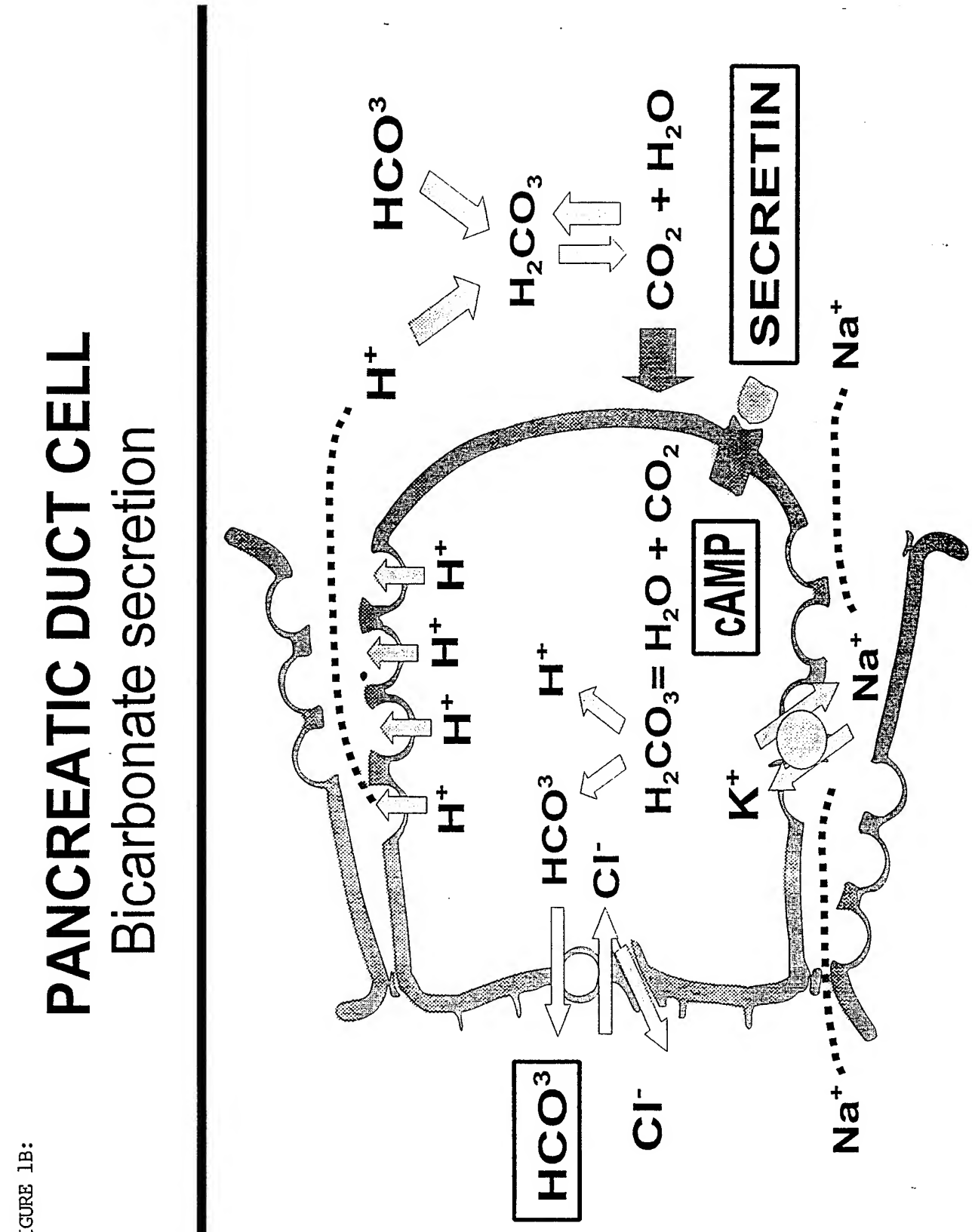

PANCREATIC DUCT CELL
Bicarbonate secretion
HCO3
H+
H2CO3
CO2 + H2O
SECRETIN
H+ H+ H+ H+
H+
HCO3
Cl-
H2CO3= H2O + CO2
cAMP
K+
Na+
Na+
HCO3
Cl-
Na+

FIGURE 1C:

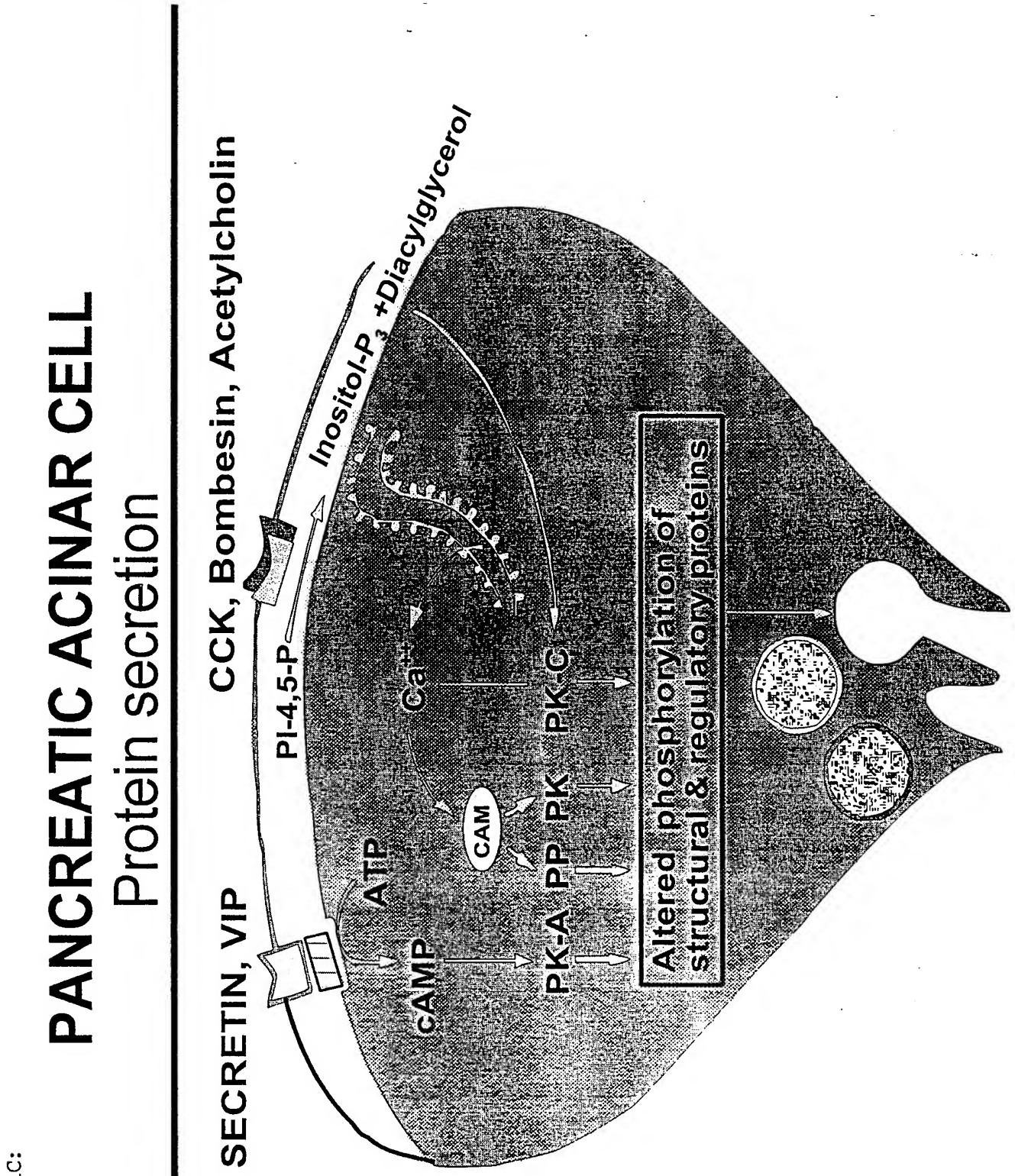

PANCREATIC ACINAR CELL
Protein secretion
SECRETIN, VIP
CCK, Bombesin, Acetylcholin
PI-4,5-P
Inositol-P3 +Diacylglycerol
ATP
cAMP
Ca++
CAM
PK-A PP PK
PK-C
Altered phosphorylation of structural & regulatory proteins

FIGURE 2:

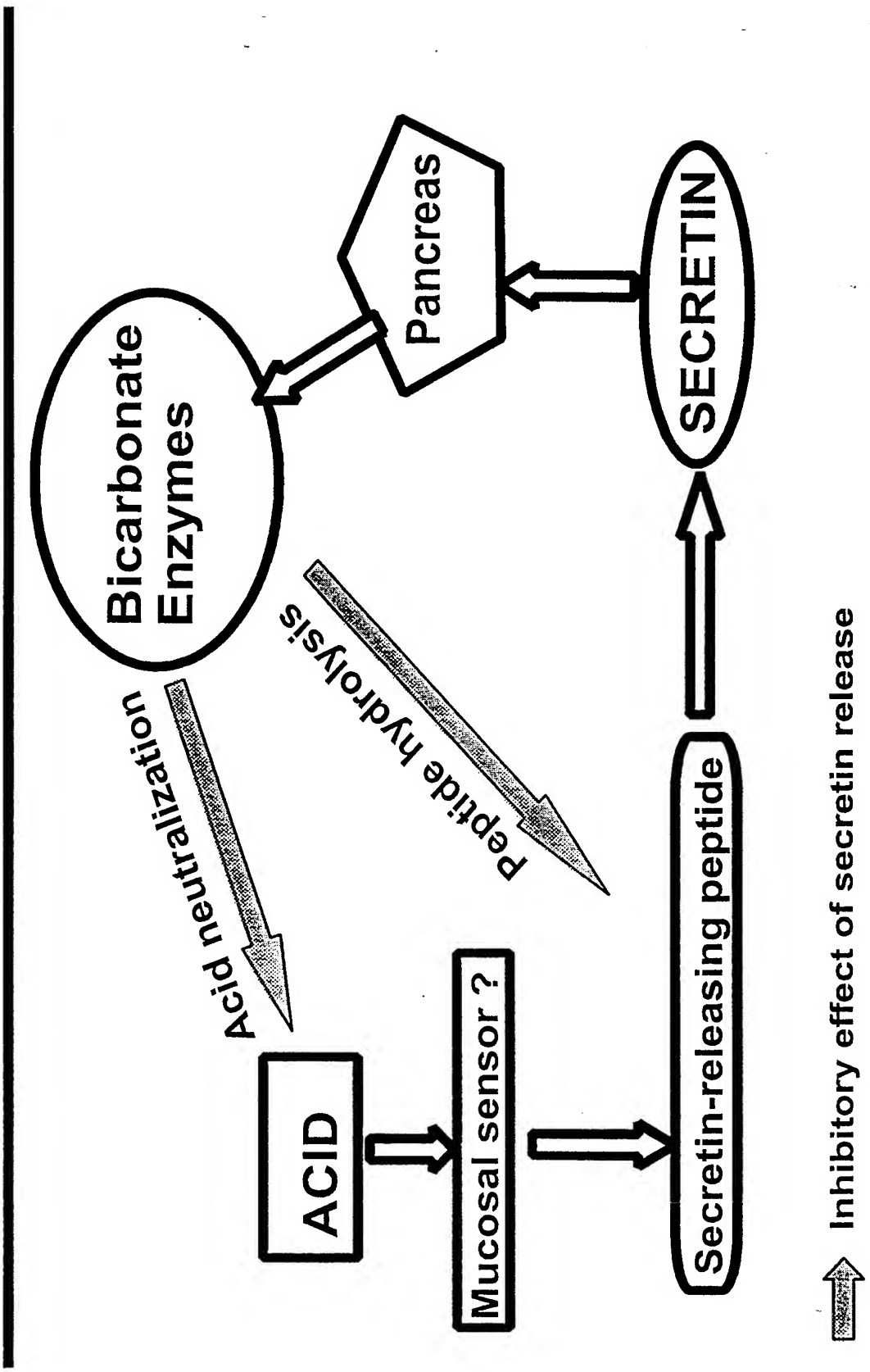
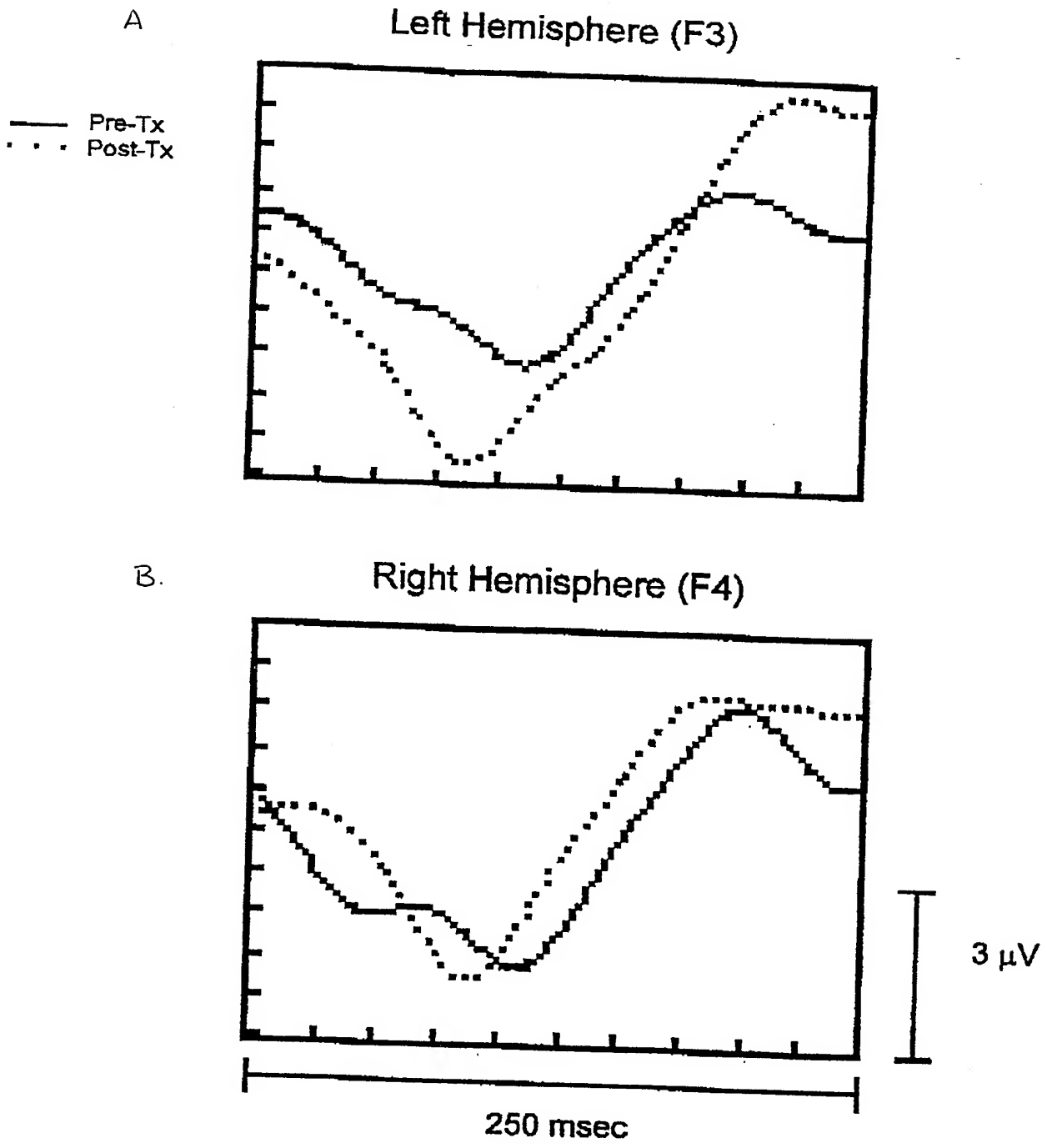

FEED-BACK MECHANISM OF SECRETIN RELEASE
Bicarbonate Enzymes
Pancreas
SECRETIN
Acid neutralization
Peptide hydrolysis
ACID
Mucosal sensor ?
Secretin-releasing peptide
Inhibitory effect of secretin release

FIGURE 3:


A
Left Hemisphere (F3)
Pre-Tx
Post-Tx
B.
Right Hemisphere (F4)
3 μV
250 msec

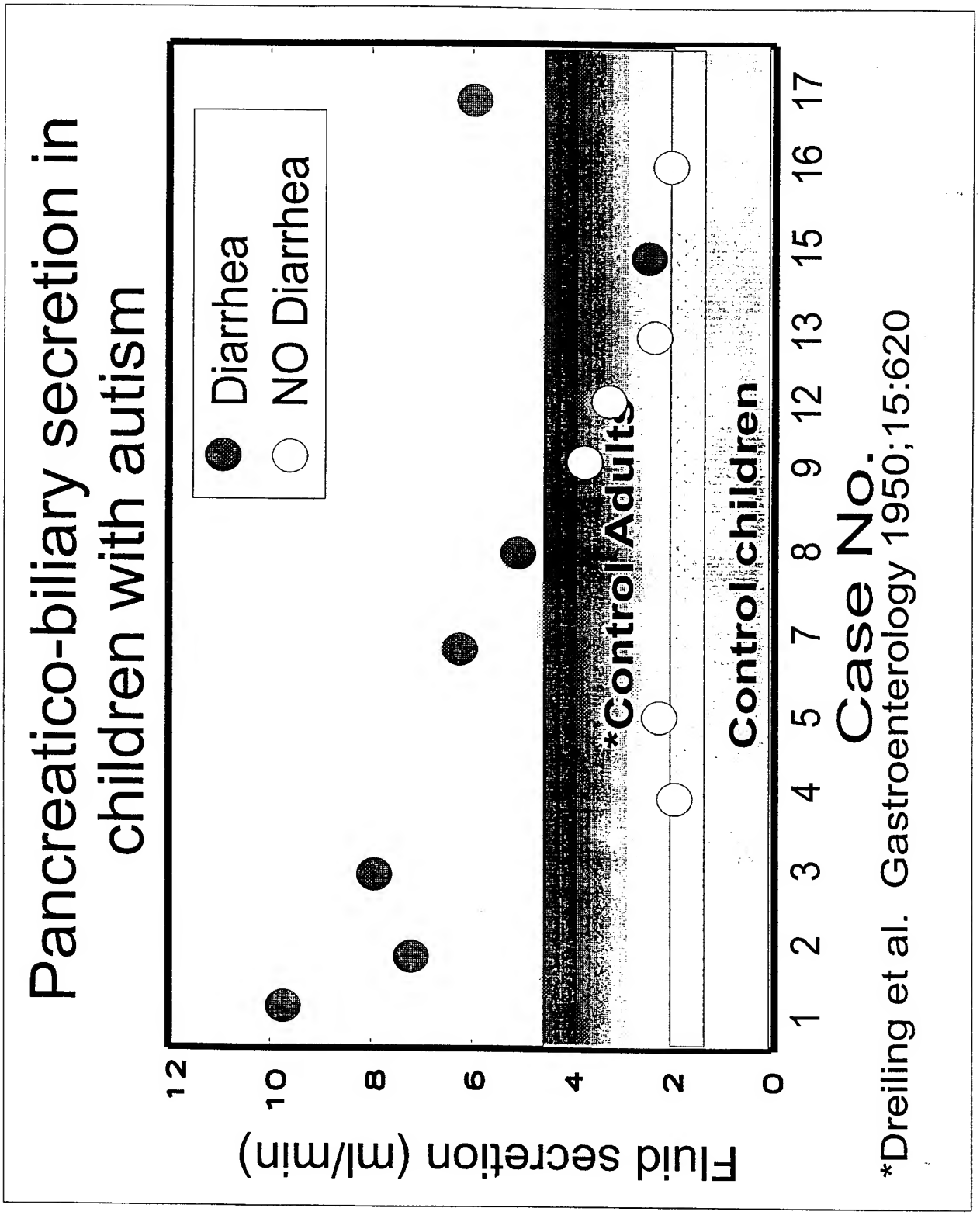

Pancreatico-biliary secretion in children with autism
Diarrhea
NO Diarrhea
*Control Adults
Control children
Fluid secretion (ml/min)
0
2
4
6
8
10
12
1 2 3 4 5 7 8 9 12 13 15 16 17
Case No.
*Dreiling et al. Gastroenterology 1950;15:620

FIGURE 4:

Figure 5. BLOOD SECRETIN CONCENTRATIONS IN AUTISTIC CHILDREN

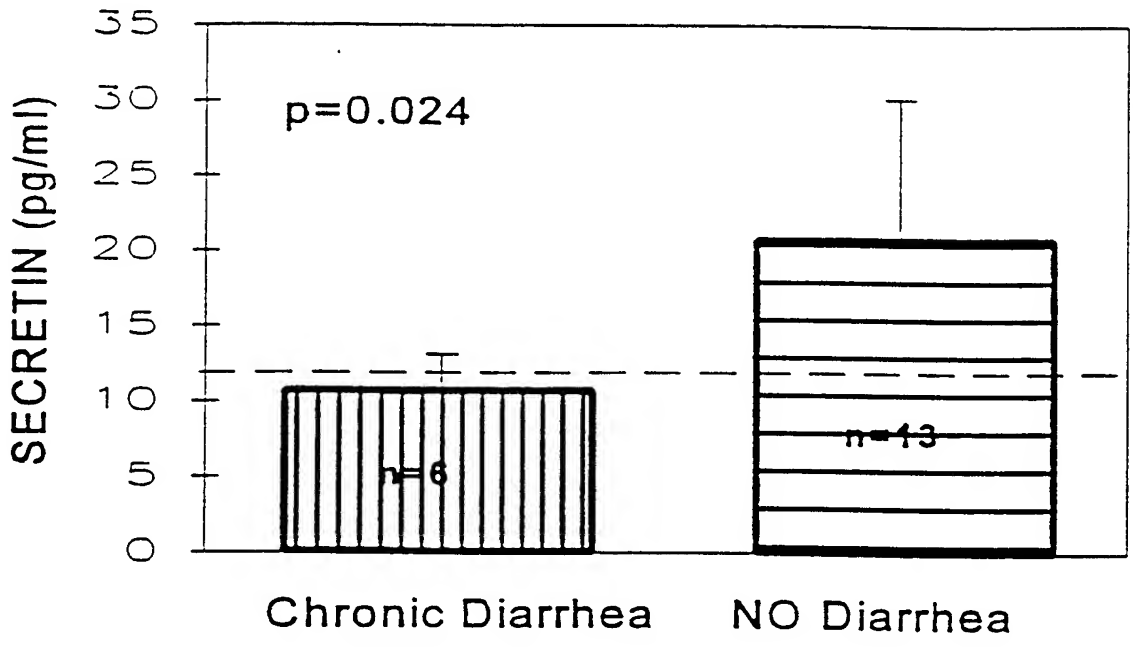
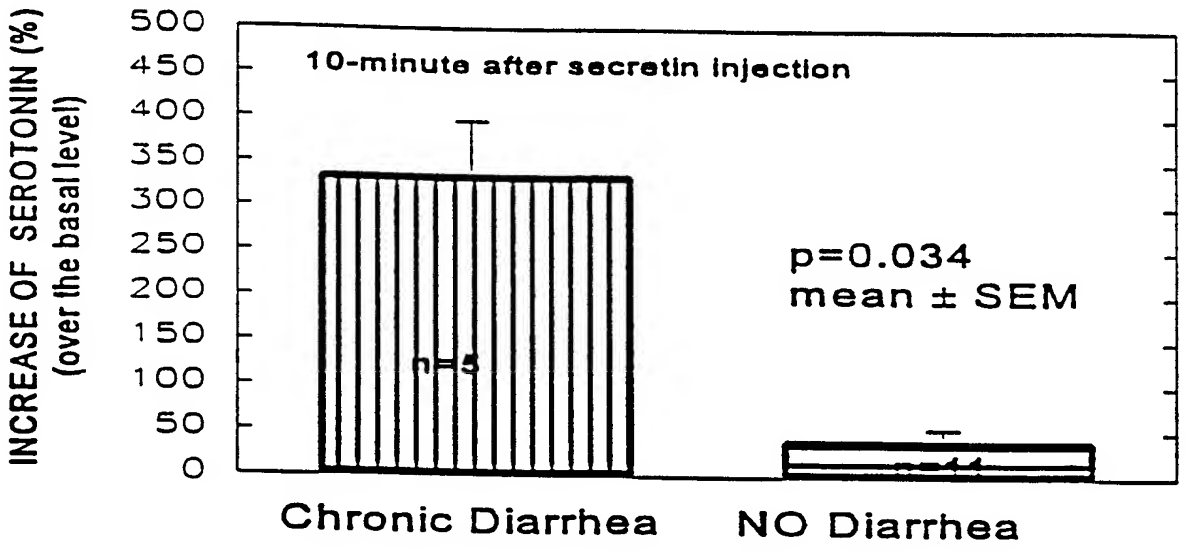


Figure 6. CHANGES IN BLOOD SEROTONIN CONCENTRATIONS IN AUTISTIC CHILDREN AFTER SECRETIN INJECTION



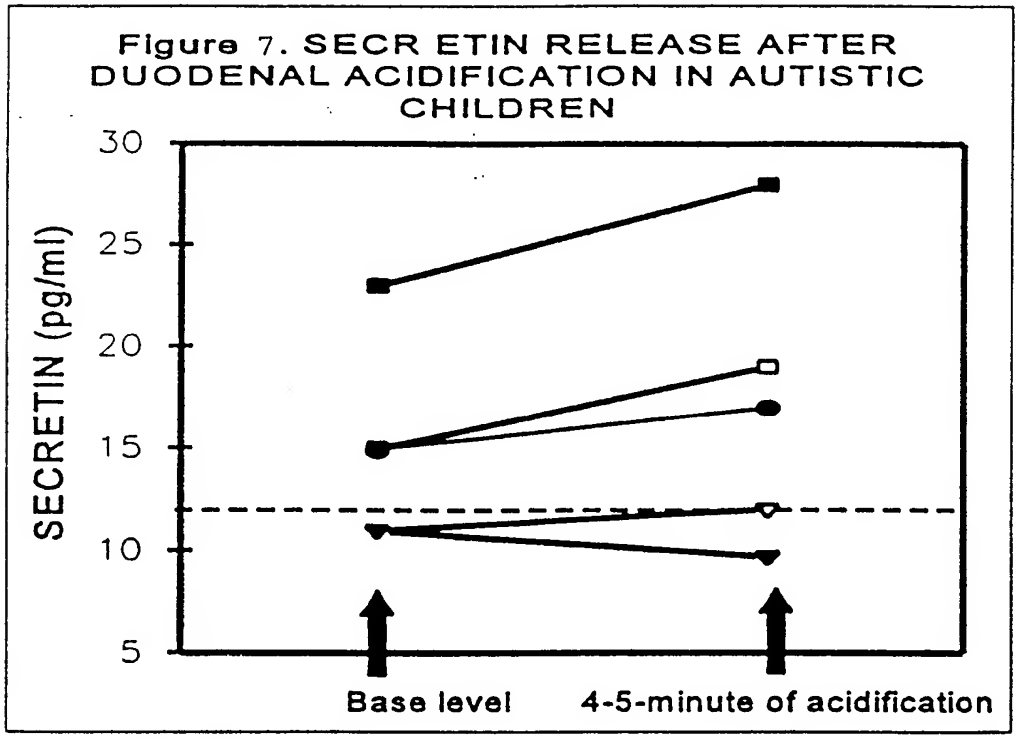


Figure 7. SECR ETIN RELEASE AFTER DUODENAL ACIDIFICATION IN AUTISTIC CHILDREN
SECRETIN (pg/ml)
30
25
20
15
10
5
Base level
4-5-minute of acidification

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/10224

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :424/184.1, 198.1; 514/2-21, 254; 436/501
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/184.1, 198.1; 514/2-21, 254; 436/501

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,778,794 A (NARUSE et al) 18 October 1988, see entire document.	1-20
A	COOK et al. The serotonin system in autism. Current Opinion in Pediatrics. 1996, Vol. 8, pages 348-354, see entire document.	1-20
A,P	HARTEVELD et al. Autism: Role of drug treatment and a guide to its use. CNS Drugs. September 1997, Vol. 8, No. 3, pages 227-236, see entire document.	1-20
A	RITVO et al. A medical model of autism: Etiology, Pathology and Treatment. Pediatric Annals. 1984, Vol. 13, No. 4, pages 298-305, see entire document.	1-20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
13 OCTOBER 1998	23 OCT 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer NITA M. MINNIFIELD Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/10224

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,920,122 A (NARUSE et al) 24 April 1990, see entire document.	1-20

INTERNATIONAL SEARCH REPORT

Intern. ional application No.
PCT/US98/10224

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 38/00, 38/28, 38/16 39/00, 39/38, 31/495, 31/50; G01N 33/566; A01N 37/18, 43/58, 43/60

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

EMBASE, BIOSIS, MEDLINE, BIOTEHCDS, JAPIO, APS, WPIDS, CABA, AGRICOLA, LIFESCI, CONFSCI, DISSABS, CAPLUS

search terms: inventor names, autism, autistic syndrome, screening, diagnosis, serotonin, secretin, treatment, secretin release stimulating substance, pancreatico-biliary fluid, cerebellum